# UNITED STATES PATENT AND TRADEMARK OFFICE

## BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte FRANKLIN H. PORTUGAL, RITA R. COLWELL
ANWARUL HUQ and AFZAL CHOWDHURY



Appeal No. 2004-1967
Application No. 09/027,439

ON BRIEF

Before SCHEINER, GRIMES and GREEN, Administrative Patent Judges.

SCHEINER, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the final rejection of claims 47, 48, 53 and 55-58. Claims 21-36 and 52 are also pending, but claims 21-26 have been withdrawn from consideration, while claim 52 has been allowed.

BACKGROUND

Shigellosis, also known as bacillary dysentery, is caused by four major species of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Specification, pages 2 and 3. "Reiter's disease, reactive arthritis, and hemolytic uremic syndrome are possible sequelae . . . of shigellosis." Id. "Enterohemorrhagic *E. coli* (EHEC) is a defined subset of toxin-producing *Shigella* and at least one serotype (0157:H7) of EHEC can cause hemorrhagic colitis and hemolytic uremic syndrome, a potentially fatal complication." Id., page 3.

"[I]nfection [with *Shigella* or enterohemorrhagic *E. coli*] is a serious public health problem in the United States and [ ] physicians are required to report cases to the Centers for Disease Control and Prevention." Id., page 2. "Although the Centers for Disease Control require distinguishing among the four major species of *Shigella* in its reporting statistics, it is difficult to do so." Id., page 4. Moreover, "[t]he genus *Shigella* is a member of the family of *Enterobacteriaceae* and is thus related to *Escherichia coli.* [*Shigella* and *Escherichia*] DNA relatedness is very high, they are often difficult to differentiate biochemically, and they cross-react serologically." Id., page 3.

"Distinguishing between *Shigella* and *E. coli* is important for treating [ ] infection." Id., page 4. The present invention is directed to "species-specific and genus-specific and, therefore, species-identifying and genus-identifying, nucleotides of [16s ribosomal RNA and 16s ribosomal DNA] from *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii*, and *Shigella dysenteriae*" (id., page 5), which can be used to "distinguish[ ] *Shigella* from *E. coli* and species of *Shigella* from each other." Id. SEQ ID NOS: 3, 4 , 5 and 6 represent the nucleotide sequences of 16s rDNA of *S. flexneri*, *S. sonnei*, *S. dysenteriae* and *S. boydii*, respectively. Id., page 12. Finally, exemplary genus-specific and species-specific probes are set forth in Table 3 of the specification.

## THE CLAIMS[1]

Claims 55-58 are directed to nucleic acid probes which target individual species of *Shigella*. According to the specification, probes are "synthetic or biologically produced nucleic acids that . . . contain specific . . . sequences that allow them to hybridize under defined predetermined stringencies, specifically and preferentially to

[1] Following some discussion on the record, appellants and the examiner agreed that the Appendix accompanying the Substitute Appeal Brief dated February 2, 2004 sets forth the correct claims on appeal.

target nucleic acid sequences . . . [wherein] a target nucleic acid sequence contains a species-specific or genus specific nucleotide." Specification, page 17. Targeting probes are "complementary to areas of rRNA or rDNA having species-specific nucleotides or genus-specific nucleotides" and are capable of "detecting and discriminating . . . the presence of rRNA or rDNA molecules of *Shigella* species and *E. coli*." Id., page 13. Claims 55 and 56 are representative (differences underlined):

55. A probe which
a) targets *Shigella flexneri* comprising a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 3, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules,
b) targets *Shigella sonnei* comprising a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 4, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules,
c) targets *Shigella dysenteriae* comprising a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 5, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules, or
d) targets *Shigella boydii* comprising a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 6, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules.

56. A probe which
a) targets *Shigella flexneri* consisting of a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 3, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules,
b) targets *Shigella sonnei* consisting of a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 4, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules,
c) targets *Shigella dysenteriae* consisting of a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 5, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules, or
d) targets *Shigella boydii* consisting of a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 6, an RNA equivalent thereof, or a nucleic acid complementary to said molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules.

Claims 47, 48 and 53 are directed to isolated nucleic acid molecules comprising or consisting of SEQ ID NO: 3, 4, 5 or 6; RNA equivalents of SEQ ID NO: 3, 4, 5 or 6; and complements of SEQ ID NO: 3, 4, 5 or 6. Claims 47 and 48 distinguish between "complementary" molecules "capable of base pairing [with molecules comprising or consisting of SEQ ID NO: 3, 4, 5 or 6] according to the standard Watson-Crick complementarity rules" (i.e., A to T or U, and G to C) and "substantially complementary" molecules "capable of hybridizing" to SEQ ID NO: 3, 4, 5 or 6 under specified conditions. Ostensibly, substantially complementary nucleic acids are something other than completely complementary – mismatches can be tolerated, as long as the molecule is capable of hybridizing to SEQ ID NO: 3, 4, 5 or 6 under the stated conditions. Claims 47, 48 and 53 read as follows:

47. An isolated nucleic acid molecule comprising SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6,
or an RNA equivalent thereof,
or a nucleic acid complementary to said isolated molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules,
or a nucleic acid substantially complementary to said isolated molecule which is capable of hybridizing to the nucleic acid molecule under the following stringent conditions:
hybridization at 40°-65°C for 14-16 hours in a hybridization solution at pH 7.8, containing 0.9 M NaCl, 0.12 M Tris-HCl, 6 nM EDTA, 0.1 M sodium phosphate buffer, 0.1% SDS and 0.1% polyvinylpyrrolidone,
followed by three 15-minute washes at 40°-65°C to remove unbound probes in a solution at pH 7, containing 0.075 M NaCl, 0.0075 M Na Citrate and 0.1% SDS.

48. An isolated nucleic acid molecule consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6,
or an RNA equivalent thereof,
or a nucleic acid complementary to said isolated molecule, capable of base-pairing according to the standard Watson-Crick complementarity rules,
or a nucleic acid substantially complementary to said isolated molecule which is capable of hybridizing to the nucleic acid molecule under the following stringent conditions:

hybridization at 40°-65°C for 14-16 hours in a hybridization solution at pH 7.8, containing 0.9 M NaCl, 0.12 M Tris-HCl, 6 nM EDTA, 0.1 M sodium phosphate buffer, 0.1% SDS and 0.1% polyvinylpyrrolidone,

followed by three 15-minute washes at 40°-65°C to remove unbound probes in a solution at pH 7, containing 0.075 M NaCl, 0.0075 M Na Citrate and 0.1% SDS.

53. An isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6,

or an RNA equivalent thereof,

or a nucleic acid complementary to said isolated molecule, capable of base-pairing according to standard Watson-Crick complementarity rules.

The claims stand rejected as follows:

I. Claims 55-58 under 35 U.S.C. § 112, second paragraph, as indefinite.

II. Claims 55-58 under 35 U.S.C. § 101 as "directed to non-statutory subject matter.

III. Claims 47, 48, 53 and 55-58 under 35 U.S.C. § 102(a) and (e) as anticipated by Hogan.[2]

IV. Claims 47, 48, 53 and 55-58 under 35 U.S.C. § 102(a) as anticipated by Genbank Accession No. X96964[3] or X80726[4] (disclosed in Cilia[5]).

V. Claims 47, 48, 53 and 55-58 under 35 U.S.C. § 103 as unpatentable over Genbank Accession No. A14565[6] and Dyson.[7]

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[2] Hogan et al., U.S. Patent 5,541,308, issued July 30, 1996.

[3] Genbank Accession No. X96964, February 4, 1996.

[4] Genbank Accession No. X80726, March 29, 1996.

[5] Cilia et al., "Sequence Heterogeneities Among 16S Ribosomal RNA Sequences, and Their Effect on Phylogenetic Analyses at the Species Level," Mol. Biol. Evol., Vol. 13, No. 3, pp. 451-461 (February 20, 1996).

[6] Genbank Accession No. A14565, September 29, 1994.

[7] Dyson, N.J., in Essential Molecular Biology Vol. II: A Practical Approach, Chapter 5, pp. 11-156, Brown, T.A. ed., Oxford University Press, 1992.

## DISCUSSION

I. Indefiniteness

"[T]he definiteness of the language employed [in a claim] must be analyzed - - not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971).

Claim 55 is directed to a probe which targets *Shigella flexneri*, *S. sonnei*, *S. dysenteriae* or *S. boydii*, "comprising a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO:3," SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, respectively. Claim 56 is directed to similar subject matter, except that the probe "consist[s] of a fragment greater than 10 to 40 bases in length of a nucleotide sequence SEQ ID NO: 3," SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. According to the examiner, claims 55-58 are indefinite because the recitation "greater than 10 to 40 bases in length" "can be interpreted a number of different ways." Answer, page 6. For example "[t]he claim can be interpreted to encompass greater than '10 to 40' bases . . . [thus] 50 nucleic acid[s] would be greater than '10 to 40' but 15 would not be greater than '10 to 40' because 15 nucleic acid[s] would be within the range of 10-40, not greater than the range. The claim could also be interpreted to encompass any number of lower length limitations . . . [but] no upper length limitations." Id.

Nevertheless, we agree with appellants that the only reasonable interpretation of the phrase "greater than 10 to 40 bases in length" is that it "define[s] a range having a lower limit of greater than 10 bases in length, i.e. 11 bases in length, and an upper limit

of 40 bases in length." Brief, page 5. This interpretation is entirely consistent with the specification, which teaches that probes "between about 10 and about 40 nucleotides" in length are appropriate for detection of species-specific or genus-specific nucleotides by hybridization, while "probes having complementary regions of 15 to 25 nucleotides are most preferable." Specification, page 16. In addition, while we agree with the examiner that "the use of the term 'comprising' [in claim 55] . . . encompasses sequences on either side of the defined sequence" (Answer, page 6), we do not agree that it makes the claim indefinite. As explained in the specification, probes "may include additional residues, such as additional 5' or 3' nucleotides . . . so long as the sequence meets the [required] criteria" (Specification, page 13), in this case, targeting the appropriate species of *Shigella*. In addition, "[i]ndicator molecules . . . may be attached to nucleic acid molecules . . . at the 3' end, the 5' end, or at other locations on the molecule" (id., page 14).

The examiner also criticizes the term "said molecule" in claims 55 and 56 as lacking sufficient antecedent basis. However, we are persuaded that one skilled in the art would understand that "said molecule" refers to the "fragment . . . of [ ] nucleotide sequence SEQ ID NO: 3," 4, 5 or 6.

The rejection of claims 55-58 under the second paragraph of 35 U.S.C. § 112, is reversed.

II. Statutory Subject Matter under 35 U.S.C. § 101

According to the examiner, claims 55-58 "do not sufficiently distinguish over nucleic acids as they exist naturally because the claims do not particularly point out any non-naturally occurring differences between the claimed products and the naturally

occurring products." Answer, page 4. Nevertheless, these claims are directed to probes that target individual *Shigella* species. As discussed above, "a target nucleic acid sequence contains a species-specific or genus specific nucleotide" (Specification, page 17) and targeting probes are "complementary to areas of rRNA or rDNA having species-specific nucleotides or genus-specific nucleotides" and are capable of "detecting and discriminating . . . the presence of rRNA or rDNA molecules of *Shigella* species" (id., page 13).

The examiner has provided no evidence of any naturally occurring product capable of targeting (i.e., distinguishing between) *Shigella flexneri*, *S. sonnei*, *S. dysenteriae* and *S. boydii*, as required by the claims. The rejection of claims 55-58 under 35 U.S.C. § 101 is reversed.

III. Anticipation by Hogan

"[E]very limitation of a claim must identically appear in a single prior art reference for it to anticipate the claim." Gechter v. Davidson, 116 F.3d 1454, 1457, 43 USPQ2d 1030, 1032 (Fed. Cir. 1997). Moreover, "the Patent Office has the initial burden of coming forward with some sort of evidence tending to disprove novelty." In re Wilder, 429 F.2d 447, 450, 166 USPQ2d 545, 548 (CCPA 1970).

Claims 47, 48, 53 and 55-58 stand rejected under 35 U.S.C.§ 102 (a) and (e) as anticipated by Hogan. According to the examiner, Hogan describes "a probe which detects *E. coli* . . . (hereinafter termed 'Sequence A') which is completely complementary to positions 991-1120 of SEQ ID NO[:] 3, and positions 989-1018 of SEQ ID NO[:] 6 . . . [and] also has complementary sequences to both SEQ ID NO[:] 4, over positions 990-1019, and SEQ ID NO[:] 5 over positions 990-1019. Hogan teaches

another sequence . . . (hereinafter termed 'Sequence B') which is completely complementary to positions 955-993 of SEQ ID NO[:] 3, positions 954-992 of SEQ ID NO[:] 5, and positions 953-991 of SEQ ID NO[:] 6." Answer, page 7. Nevertheless, we agree with appellants that neither of Hogan's probes anticipates the subject matter of claims 47, 48, 53 or 55-58.

Claims 47, 48 and 53 are directed to nucleic acid molecules comprising or consisting of SEQ ID NO: 3, 4, 5 or 6; RNA equivalents of SEQ ID NO: 3, 4, 5 or 6; and either complete or substantial complements of SEQ ID NO: 3, 4, 5 or 6. SEQ ID NOS: 3, 4, 5 and 6 are 1506, 1505, 1453 and 1505 bases in length, respectively. Hogan's Sequences A and B are 30 and 39 bases in length, respectively. Therefore, neither Sequence A nor Sequence B is long enough to meet the requirements of claim 47, 48 or 53. We see no basis for the examiner's assertion that complete or substantial complements of SEQ ID NO: 3, 4, 5 or 6 need not extend the full length of SEQ ID NO: 3, 4, 5 or 6 to anticipate the claims, and that "the sequences of Hogan meet the claim requirements because they are [ ] completely complementary to regions within SEQ ID NOS[:] 3, 5 and 6." Answer, page 10. To be "equivalent," "complementary" or "substantially complementary," to SEQ ID NO: 3, 4, 5 or 6, a molecule must extend the full length of SEQ ID NO: 3, 4, 5 or 6, not just a portion of it. The requirement that the molecule be capable of base-pairing with SEQ ID NO: 3, 4, 5 or 6 according to standard Watson-Crick rules (i.e., completely complementary), or capable of hybridizing to SEQ ID NO: 3, 4, 5 or 6 under stringent conditions (i.e., substantially complementary), is an additional, not alternative, limitation.

Claims 55-58 are directed to a probe comprising or consisting of a fragment of SEQ ID NO: 3, 4, 5 or 6; an RNA equivalent of the fragment of SEQ ID NO: 3, 4, 5 or 6; or a complement of the fragment of SEQ ID NO: 3, 4, 5 or 6; wherein the probe "targets" *Shigella flexneri*, *S. sonnei*, *S. dysenteriae* or *S. boydii*. As explained in the specification, targeting probes are "complementary to areas of rRNA or rDNA having species-specific nucleotides or genus-specific nucleotides" and are capable of "detecting and discriminating . . . the presence of rRNA or rDNA molecules of *Shigella* species and *E. coli*." Specification, page 13. Hogan's probes may be the same length as the claimed probes, but they hybridize with nucleic acid from *E. coli*, *S. boydii*, *S. flexneri*, and *S. sonnei* (see Table 54 of Hogan), thus, they are not capable of distinguishing between individual species of *Shigella*. That is, neither of Hogan's probes is capable of targeting and discriminating between individual species of *Shigella*, as required by the claims. We see no basis for the examiner's assertion that "'targets' . . . is broadly interpreted to encompass probes which will hybridize to or detect species of *Shigella*." Answer, page 8.

The rejection of claims 47, 48, 53 and 55-58 under 35 U.S.C. § 102 (a) and (b) as anticipated by Hogan is reversed.

IV. Anticipation by Genbank Accession No. X96964 or X80726

Claims 47, 48, 53 and 55-58 stand rejected under 35 U.S.C.§ 102 (a) as anticipated by Genbank Accession No. X96964 or X80726, both of which set forth DNA sequences described as encoding the *S. sonnei* 16S ribosomal RNA. It may be, as the examiner asserts, that "the complements of the accession numbers are [ ] 'substantially complementary' to [SEQ ID NO: 4] and would be capable of hybridizing to [SEQ ID NO: 4] under the recited conditions." Answer, page 11. Nevertheless, SEQ ID NO: 4 is

1505 bases in length, while Accession Nos. X96964 and X80726 are 1488 and 1467 bases in length, respectively. Id. Therefore, neither is long enough to meet the requirements of claim 47 or 48. With respect to claim 53, the prior art sequences are not only too short to meet the requirements of the claim, they contain 5 and 10 mismatches, respectively. Answer, pages 11-12. The examiner has not established that either prior art molecule would be "capable of base-pairing according to the standard Watson-Crick complementarity rules," as required by the claim. Again, as discussed above, we see no basis for the examiner's assertion that complete or substantial complements of SEQ ID NO: 3, 4, 5 or 6 need not extend the full length of SEQ ID NO: 3, 4, 5 or 6 to anticipate the claims.

With respect to claims 55-56, the examiner has not established that either of the prior art sequences would be capable of specifically targeting *S. sonnei*, given the mismatches between the prior art and claimed sequences.

The rejection of claims 47, 48, 53 and 55-58 under 35 U.S.C.§ 102 (a) as anticipated by Genbank Accession No. X96964 or X80726 is reversed.

V. Obviousness

Claims 47, 48, 53 and 55-58 stand rejected under 35 U.S.C.§ 103 (a) as unpatentable over Genbank Accession No. A14565 in view of Dyson. We reverse this rejection with respect to claims 48, 53 and 55-58, but affirm it with respect to claim 47.

Genbank Accession No. A14565 sets forth the DNA sequence encoding the 16S ribosomal RNA of *E. coli*. With respect to claim 48, Accession No. A14565 is 1541 bases in length, and is therefore too long to be "an isolated nucleic acid molecule consisting of SEQ ID NO:3" (emphasis added). With respect to claim 53, the extra nucleotide residues are of no moment because the claim uses open language, but

there are five mismatches and one gap between the sequences of A14565 and SEQ ID NO:3 (Answer, page 14), thus A14565 is not "an isolated molecule comprising SEQ ID NO:3" (emphasis added) – nor would its complement be capable of base-pairing to SEQ ID NO:3 according to the Watson-Crick rules of complementarity. With respect to claims 55-58, A14565 represents the sequence of *E. coli* 16S rRNA, and would not appear to be capable of targeting particular species of *Shigella*.

The examiner has not recognized any of these differences between A14565 and the subject matter of claims 48, 53 and 55-58 and has not identified any reason to modify A14565 to meet the specific limitations of these claims. Nevertheless, the initial burden of unpatentability rests on the examiner (In re Oetiker, 977 F.2d 1443, 1446, 24 USPQ2d 1443, 1445 (Fed. Cir. 1992)), and it is well established that there must be a reason, suggestion or motivation to lead an inventor to modify a reference in the manner claimed (Pro-Mold & Tool Co. v.Great Lakes Plastics, Inc., 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996)).

Accordingly, the rejection of claims 48, 53 and 55-58 under 35 U.S.C.§ 103 (a) is reversed.

Claim 47 stands on a different footing. Claim 47 is directed in part to "a nucleic acid substantially complementary to [an isolated nucleic acid molecule comprising SEQ ID NO: 3] which is capable of hybridizing to the nucleic acid molecule under [specified] stringent conditions[.]" The examiner notes that the specification teaches that mismatches can be tolerated under the claimed hybridization conditions, and also that the effect of mismatches decreases with increasing probe length. Answer, page 14. According to the examiner, "it would have been prima facie obvious for one skilled in the art to construct the complement of accession number A14565 . . . for the purpose[ ]

of detecting accession number A14565" since "[s]uch methods are . . . exemplified by Dyson" (Answer, page 14), and "the complement of [ ] accession number [A14565] would hybridize to SEQ ID NO[:] 3 because the effect of the [5] mismatches would be diminished due to the length of the [molecule]." Id.

Appellants argue only that the complement of Accession No. A14565 is not disclosed in the reference, and that no evidence of motivation to prepare a complementary sequence has been presented. Brief, page 7. This argument is not persuasive. We think it is fair to say that a description of any single-stranded nucleic acid is a constructive description of its complement, as it is made from – or serves as a template for – a complementary strand of nucleic acid. In our opinion, Accession Number A14565 anticipates the subject matter of claim 47 (and anticipation is the epitome of obviousness, In re May, 574 F.2d 1082, 1089, 197 USPQ 601, 607 (CCPA 1978)). Even if this were not the case, however, the examiner cited Dyson as evidence of a motivation to prepare the complement of accession number A14565, yet appellants did not address this evidence in any way. We hold that the examiner has established a prima facie case of obviousness with respect to claim 47, which appellants have not overcome by evidence or argument.

The rejection of claim 47 under 35 U.S.C. § 103 (a) is affirmed.

## CONCLUSION

We affirm the examiner's rejection of claim 47 under 35 U.S.C. § 103 (a), but reverse the rejection with respect to claims 48, 53 and 55-58. In addition, we reverse the rejections of claims 47, 48, 53 and 55-58 under 35 U.S.C. §§ 101; 112, second paragraph; and 102 (a) and (e).

## AFFIRMED-IN-PART

Toni R. Scheiner
Administrative Patent Judge

Eric Grimes
Administrative Patent Judge

Lora M. Green
Administrative Patent Judge

) BOARD OF PATENT
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Millen, White, Zelano & Branigan, P.C.
2200 Clarendon Blvd, Suite 1400
Arlington, VA 22201